

NATIONAL STANDARD METHOD

INOCULATION OF CULTURE MEDIA

QSOP 52

Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections



Association of Medical Microbiologists
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STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

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The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

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AMENDMENT PROCEDURE

Controlled document reference	QSOP52
Controlled document title	Inoculation of culture media

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
			All	All	This document was formerly BSOP 54 but has been changed to a QSOP to which it is better suited. Changes below are the differences between BSOP 54 and QSOP 52
			1	Front page	Northern Ireland logo added
			All	All	Hyperlinks to relevant NSMs inserted
			11	Diagrams	Revised and improved
			12	References	References reviewed and updated

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INOCULATION OF CULTURE MEDIA

SCOPE OF DOCUMENT

This NSM describes the basic methods of inoculating culture media with specimens, and sub-culturing of organisms from one medium to another.

INTRODUCTION

To process clinical specimens satisfactorily for bacteriological culture, consideration must be given to^{2,3}:

- Samples (where possible) are taken before antimicrobial therapy is started
- The need to process specimens within appropriate time scale for organism viability and clinical need
- The safety aspects of specimen processing
- The specimen type and its anatomical origin
- The requirement for pre-treatment (eg centrifugation, homogenisation, dilution etc)
- The selection of primary isolation media
- The incubation temperature and atmosphere

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1 GENERAL PRINCIPLES

In general media should be inoculated in a logical order from least selective to most selective to avoid the inhibition of organisms by the selective agent:

- 1st - media without inhibitors (eg blood agar)
- 2nd - indicator media (eg CLED agar)
- 3rd - selective media (eg XLD agar, GC selective agar)
- 4th - smears for staining

There may be occasions where it may not be advisable to inoculate media in this way. For example, swabs for gonococcal (GC) culture may contain only small numbers of organisms. This will make the inoculation of the GC selective agar the priority. Where specimens are insufficient for a full range of culture plates, priorities should be based on origin of specimen and the range of likely organisms to be encountered.

Liquid media may be inoculated first when processing fluid specimens. This reduces the chances of carry-over from contaminated solid media. However, liquid media should be inoculated after the solid media when swabs and faeces are examined, to avoid diluting the organisms contained on or in the sample and to avoid any non-viable organisms present in a liquid medium being transferred to other liquid media, solid media or to slides.

Smears for staining are usually made after all culture media have been inoculated to avoid carry-over of contaminants that may be on the surface of the slide. However, there may be occasions where the smear is required prior to culture and then a sterile slide should be used. Slides may be sterilised by flooding the slide with alcohol, discarding the excess and drying on a hotplate. Under no circumstance should the alcohol be burned off in a Bunsen flame.

For the isolation of individual colonies, the inoculum should be spread, usually by using a sterile loop, taking care to avoid the edges of the plate where contaminants are more likely to be located.

Antimicrobial discs for identification (eg optochin, bacitracin) may be added as appropriate. Discs should be placed near the edge of the plate, between the areas covered by the first and second spread, to avoid total inhibition of very susceptible organisms.

As a minimum requirement, all culture plates and containers must be labelled to identify the patient name or laboratory number or barcode. Additional labelling, including date of culture or sub-culture will be necessary for selected specimens, such as those requiring prolonged incubation or sub-culture from enrichment broth.

To work safely and minimise risks of cross contamination suitable racks should also be used when inoculating, incubating or storing liquid cultures or culture plates⁴.

2 INOCULATION OF CULTURE MEDIA

For the effective detection of the bacterial content of specimens, it is important to achieve growth of individual colonies by using a good technique to inoculate the specimen on culture media. There are many variations and personal preferences for "plating out", some of which are described in this Guidance Note.

All culture media should be checked before use for contamination and expiry date. Culture media should have an identifiable batch or quality control number and have passed QC tests before use. Plates that are beyond their expiry date, contaminated plates, and broth media appearing unusually turbid should be discarded.

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The initial area inoculated should cover between a quarter and a third of the total area of agar used. Whole plates, half plates, or quarter plates can be used depending on the circumstances. Specimens may be plated out for individual colonies, or seeded directly over an entire segment of a plate and incubated without further spreading.

Wire loops should be flamed by holding them loop end down in a Bunsen flame until the loop and entire wire reach red heat. Place on a rack to cool before use. This should be done before and after use and between agar plates. It is usual to use two loops, to allow adequate cooling of one after flaming whilst the other is in use. Different disposable loops should be used for each plate.

For a potentially heavily contaminated sample, the loop should either be flamed between each series of streaks, or the loop may be rotated to make the next series of streaks with the unused side of the loop. For semi-quantitative analysis of urine, the loop should not be flamed in this way.

All media should be incubated as soon as possible after inoculation. Plates for anaerobic incubation should be incubated as soon as possible to prevent loss of viability (<15 minutes⁵).

After inoculation, the specimen, or a portion of it, should be retained for at least 48 hours after the laboratory has issued the final report⁶.

Most positive culture plates can be discarded within 24-48 hours of issuing a final authorised report. Cultures of particular epidemiological value may be retained for longer as organisms may need further work or referral to a reference laboratory⁶.

Stained routine microscope slides should be kept for seven days after issue of the final report. Slides for examination for *Mycobacterium* species should be kept locked under level 3 conditions until the final report on the specimen is issued. Positive cultures of *Mycobacterium* species should be retained in a locked cupboard in a Category 3 laboratory until the final report from the Reference Laboratory has been received.

3 ASEPTIC TECHNIQUE

When handling specimens or cultures, aseptic technique is important to avoid their contamination and to protect the worker from infection from the sample.

In-house training to demonstrate the skills of aseptic technique should be given to staff who will process specimens or cultures.

The following points should be observed when culturing specimens or performing subcultures:

- With the exception of urine specimens, caps and lids from containers should not be placed on the workbench, but retained in the hand whilst the sample is being processed, taking care not to contaminate the hand or cap. Caps and lids should be replaced as soon as possible
- If the work is being carried out on the open bench, a Bunsen burner should be in close proximity to flame loops or wires
- Keep samples away from the face when opening culture containers
- Aerosol production should be minimised by:
 - Opening caps slowly as the contents of containers are sometimes under pressure
 - Avoiding vigorous swirling or shaking of the sample prior to opening
 - Cooling loops that have been heated before use
 - Avoiding expelling the last drop from a pipette

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- If forceps or scissors are used when handling specimens, they should be heated in a Bunsen flame and allowed to cool before use
- Loops and wires should be heated in the Bunsen flame with the loop end downwards until red-hot, then removed and allowed to cool. Care should be taken that the loop does not contain fluid or large particles of matter that may 'splatter' when placed in the flame, and that the entire loop/wire up to the loop holder is heated to red-heat. A Kampf burner with a glass protective cover may be used to contain splatter

4 PRIMARY CULTURE METHODS

4.1 SWABS - PLATE CULTURE

Initial inoculum should cover between a quarter and a third of the plate to be used (Figure 1).

The swab should be rolled over the inoculation area to maximise transfer of organisms, taking care to avoid the edges of the plate.

Inoculation of samples to selective media such as Sabouraud's agar (when usually only a quarter plate will be used) may not require spreading with a loop (Figure 3).

4.2 SWABS - LIQUID CULTURE

Using aseptic technique, remove the broth container cap, place the swab in the broth, break off (or cut) the swab-stick and replace the cap. The swab may be placed in the broth directly, or after inoculating solid culture media (consideration should be given to the possibility that contaminants may be transferred into the broth from contaminated culture plates).

4.3 FLUID SPECIMENS AND PUS

The centrifuged deposit of any fluid is re suspended in approximately 0.5mL supernatant, and then transferred to the appropriate culture media with a sterile pipette. Thick pus may require inoculation with the aid of a swab/swabstick. If a semi-quantitative method is required, inoculate the media with a standard loop (1µL, 10µL etc) or a piston-operated pipette as appropriate.

4.4 URINE - CALIBRATED LOOP, SURFACE STREAK METHOD⁷

(See [BSOP 41 - Investigation of Urine](#))

The urine is mixed gently to avoid foaming.

The end of a sterile calibrated loop (eg 1µL, 2µL or 10µL) is dipped to just below the surface of the urine and removed vertically, taking care not to carry over any urine on the shank. See Figure 2.

4.5 URINE - FILTER PAPER METHOD⁸

(See [BSOP 41 - Investigation of Urine](#))

Commercially prepared sterile filter paper strip is dipped in the urine up to the mark indicated.

Remove excess urine by touching the side of the strip against the side of the container and allow the urine time to absorb into the strip before inoculating a CLED or chromogenic agar plate.

Bend the inoculated end of the strip and press it flat against the agar for a few seconds.

Several specimens may be inoculated on one CLED or chromogenic agar plate using this technique, but it is important to ensure adequate spacing to minimise the risk of any antibacterial effect.

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4.6 TISSUE AND BIOPSY SPECIMENS

Homogenise tissue with a sterile tissue grinder (Griffith's tube or unbreakable alternative) or a pestle and mortar, and inoculate 1 or 2 drops of the homogenate on appropriate media (Figure 4).

Tissue may also be cut or sliced with a sterile scalpel or, preferably, sterile scissors. Using sterile forceps, smear the sliced portion directly on the culture medium. If enrichment culture is performed to avoid possible contamination the medium should be inoculated with pieces of specimen that have not been spread over the surface of solid culture media.

All homogenisation and grinding procedures involving tissue or biopsy specimens must be performed in a Class 1 safety cabinet.

4.7 INTRAVASCULAR CANNULAE

Inoculate culture media directly by rolling the cannulae across the surface of a whole agar plate five times (avoiding the edges of the plate) or culture any blood, fluid or material contained in or on the specimen (see [BSOP 20 - Investigation of intravascular cannulae and associated specimens](#)).

5 SUBCULTURE METHODS

5.1 SUBCULTURE OF LIQUID MEDIA TO A SOLID OR LIQUID MEDIUM

Obtain samples for subculture with a sterile loop (1µL, 10µL etc) or a plastic pipette. Immerse the loop in the fluid to be subcultured, and remove carefully without allowing excess fluid to remain on the shank of the loop. Care should be taken not to contaminate the loop holder with liquid culture as this will be difficult to sterilize and may cause subsequent problems with cross contamination.

Either inoculate the loopful of fluid on an appropriate agar plate, streaking out for individual colonies (Figure 2), or inoculate 2-3 drops from the pipette on appropriate agar plates or to further fluid culture media. The use of a pipette is particularly recommended when sub-culturing organisms to multiple culture media, including those used for biochemical tests.

Subculture blood culture bottles according to manufacturer's instructions. Most continuous monitoring systems require the use of sub-vent units or sheathed needles.

NB: Caution must be observed when subculturing bottles under obvious increased pressure

Before subculture of broth that may contain mixed organisms including anaerobes, gently agitate to give an even distribution of organisms throughout the broth with as little disturbance as possible.

Subculture Selenite F and alkaline peptone water by inserting a sterile loop or pipette to the broth and sampling from just below the surface.

5.2 SUBCULTURE FROM A SOLID MEDIUM TO A LIQUID MEDIUM

Select a representative colony or colonies of the organism to be subcultured and using aseptic technique transfer to an appropriate broth with a sterile wire or loop. Gently agitate before incubation to distribute the organisms throughout the broth.

5.3 SUBCULTURE FROM A SOLID MEDIUM TO A SOLID MEDIUM

Picking colonies for subculture may be carried out with a sterile nichrome or platinum wire or loop, or a disposable equivalent. It is recommended that a sterile wire (or disposable equivalent) be used when dealing with mixed cultures to ensure the sampling of the single colonies.

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Select a representative colony or colonies of the organism to be subcultured with a sterile wire or loop, and subculture on the appropriate medium by touching the wire or loop on to the surface of the agar, and plate out.

To ensure even inoculation of biochemical test systems and multiple media, colonies should be picked and transferred to an appropriate suspension fluid or medium (eg approximately 2mL peptone water or nutrient broth). The use of a densitometer or McFarland standards may be required to adjust inoculum density. Gently agitate the suspension. Use a loopful, or a drop from a pipette of the inoculated broth, or a swab immersed in the broth suspension to inoculate the plate or test system.

The use of a pipette is recommended when subculturing fluid to more than one culture medium.

Multipoint inoculators are convenient when many replicate cultures are needed. They may be semi or fully automatic and can spot-inoculate approximately 20 cultures on a standard 9cm Petri dish, or up to 96 cultures to a microtitre tray. Multipoint inoculators have been used for urine culture, identification testing, and antimicrobial susceptibility testing. For more information refer to [BSOP 41 - Investigation of Urine](#).

Shake tube cultures are useful for observing colony formation in deep agar cultures, and are especially useful for microaerophilic and anaerobic organisms. Agar in bottles and tubes is melted and maintained at a temperature of approximately 45°C. The agar is allowed to cool slightly and a sterile loop or wire is used to inoculate the culture into the molten agar. The tube is incubated after gentle mixing. Submerged colonies will develop at different levels in the medium according to their respiratory requirements

Stab cultures can be used to observe motility, acid and gas production, and biochemical activity (eg gelatin liquefaction, DNAase). A representative colony can be picked with a sterile wire and then stabbed in the appropriate agar.

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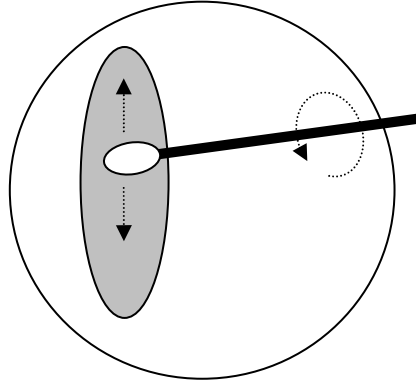
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6 ILLUSTRATION OF INOCULATION TECHNIQUE (FOR GUIDANCE ONLY)

Figure 1
Inoculation of swabs



Figures 2 and 3
Streaking inoculum for individual colonies

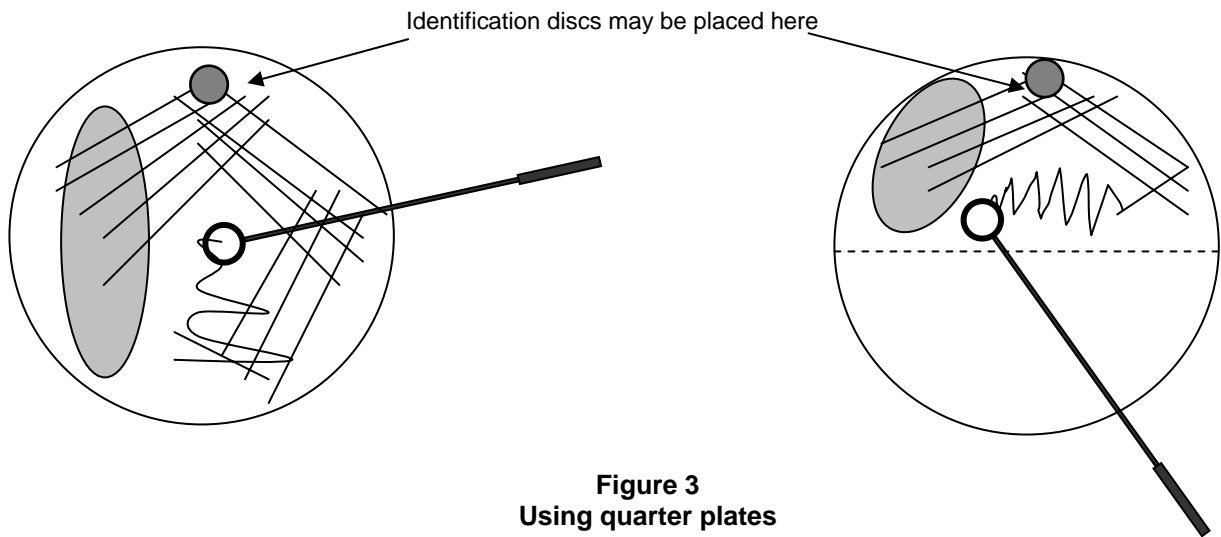
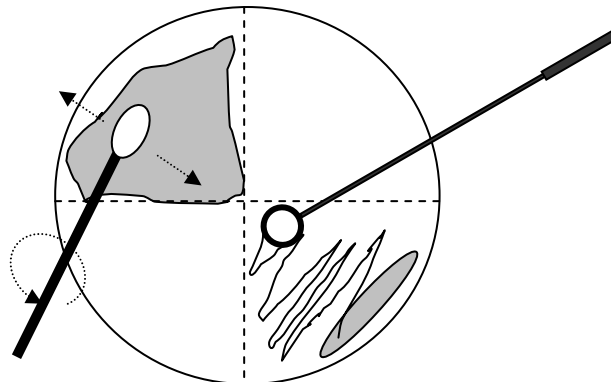


Figure 3
Using quarter plates



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7 ACKNOWLEDGEMENTS AND CONTACTS

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